

Developmental changes in fatty acid synthesis in interscapular brown adipose tissue of lean and genetically obese (*ob/ob*) mice

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Fatty acid synthesis was measured *in vivo* with $^3\text{H}_2\text{O}$ in interscapular brown adipose tissue of lean and genetically obese (*ob/ob*) mice. At 26 days of age, before the development of hyperphagia, synthesis in brown adipose tissue was higher in the obese than in the lean mice; synthesis was also elevated in the liver, white adipose tissue and carcass of the obese mice. At 8 weeks of age, when hyperphagia was well established, synthesis remained elevated in all tissues of the obese mice, with the exception of brown adipose tissue. Elevated synthesis rates were not apparent in brown adipose tissue of the obese mice at 14 days of age, nor at 35 days of age. These results demonstrate that brown adipose tissue in *ob/ob* mice has a transitory hyperlipogenesis at, and just after, weaning on to a low-fat/high-carbohydrate diet. Once hyperphagia has developed, by week 5 of life, brown adipose tissue is the only major lipogenic tissue in the obese mice not to exhibit elevated rates of fatty acid synthesis; this suggests that insulin resistance develops much more rapidly in brown adipose tissue than in other lipogenic tissues of the *ob/ob* mouse.

Brown adipose tissue is the main site of thermoregulatory non-shivering thermogenesis in the newborn of a number of mammalian species, in arousing hibernators and in cold-exposed adult rodents (Smith & Horwitz, 1969; Foster & Frydman, 1978, 1979; Thurlby & Trayhurn, 1980). Recent evidence has indicated that the tissue may also play a major role in 'regulatory' diet-induced thermogenesis (Rothwell & Stock, 1979, 1981; Brooks *et al.*, 1980). The central mechanism for heat production in brown adipose tissue is through a proton conductance pathway across the mitochondrial inner membrane, with fatty acids serving as the principal substrate (for review, see Nicholls, 1979). In adult rats and mice the capacity of brown adipose tissue for fatty acid synthesis is very high (McCormack & Denton, 1977; Trayhurn, 1979, 1981; Rath *et al.*, 1979; Agius & Williamson, 1980), the rate of synthesis in the tissue being related to the environmental temperature at which the animals are adapted (Trayhurn, 1979, 1981; Rath *et al.*, 1979); thus on a high-carbohydrate/low-fat diet, high rates of fatty acid synthesis in brown adipose tissue are associated with high rates of thermogenesis. Although the regulation of fatty acid synthesis in brown adipose tissue has been little studied, a

sensitivity to insulin (McCormack & Denton, 1977; Agius & Williamson, 1980; McCormack, 1982) and to the presence of dietary lipid (van den Brandt & Trayhurn, 1981) has been demonstrated.

Several recent studies have indicated that the thermogenic activity of brown adipose tissue is low in genetically obese mice, for both the *ob/ob* and *db/db* mutants (Himms-Hagen & Desautels, 1978; Hogan & Himms-Hagen, 1980; Thurlby & Trayhurn, 1980; Goodbody & Trayhurn, 1981, 1982). In view of the apparent relationship between thermogenesis and lipogenesis in brown adipose tissue it might be expected that fatty acid synthesis rates in the tissue would be low in genetically obese mice. In the Zucker (*fa/fa*) rat, however, it has been reported that fatty acid synthesis is greatly elevated in interscapular brown adipose tissue of 30-day-old animals, and this may reflect the effect of hyperinsulinaemia (Lavau *et al.*, 1982). We have now measured fatty acid synthesis in lean and obese *ob/ob* mice and report here a comparison of the rates obtained in interscapular brown adipose tissue and other tissues of animals aged 26 days and 8 weeks of age, i.e. before and after the development of hyperphagia (Lin *et al.*, 1977). The full developmental changes in fatty acid synthesis in brown adipose tissue in lean and obese mice are also reported.

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Materials and methods

Male mice from a colony with the 'ob' gene on the 'Aston' background were used (Thurlby & Trayhurn, 1978). They were housed in plastic cages at an ambient temperature of $22 \pm 2^\circ\text{C}$, with a 12h-light/12h-dark cycle (light period from 07:00h). After weaning at 21 days of age, lean (*ob/+* or *+/+*) and obese (*ob/ob*) littermates were caged in pairs, and fed a low-fat (3.4%, w/w)/high-carbohydrate commercial diet (Spillers–Spratts Rodent Breeding Diet 1; Spratts Patent, Barking, Essex, U.K.). Food and tap water were available *ad libitum*.

Pre-weanling *ob/ob* mice and their normal siblings were identified at 12 or 13 days of age by the 'cold-stress' test described previously (Trayhurn *et al.*, 1977). They were then marked, returned to the nest and used when aged 14 days.

Fatty acid synthesis

Rates of fatty acid synthesis were determined *in vivo* by measuring the incorporation of ^3H from $^3\text{H}_2\text{O}$ (Amersham International, Amersham, Bucks., U.K.), essentially as described previously (Trayhurn, 1980, 1981). Mice were taken fully-fed between 08:30 and 10:00h, and $400\mu\text{Ci}$ of $^3\text{H}_2\text{O}$ was injected intraperitoneally in a total volume of $75\mu\text{l}$. In the case of the pre-weanling animals $200\mu\text{Ci}$ of $^3\text{H}_2\text{O}$ in a volume of $35\mu\text{l}$ was used. At 1h after injection of radioactivity (45 min for the pre-weanling mice) blood was collected from the jugular veins, and the tissues were rapidly removed and frozen in liquid N_2 ; the remaining carcass was also frozen. Interscapular brown adipose tissue was first trimmed free of adhering connective tissue and white adipose tissue.

Blood was processed as previously described (Trayhurn, 1980). Tissues were saponified with

ethanolic KOH, acidified and fatty acids extracted by the method of Stansbie *et al.* (1976). The carcasses were autoclaved and homogenized before samples were taken for saponification (Trayhurn, 1980).

Measurement of radioactivity

Radioactivity was dissolved in a toluene/Triton X-100 (2:1, v/v)-based scintillation solution, and measured in a Packard Tri-Carb 2425 liquid-scintillation counter. The counting efficiency was approx. 25%. Corrections were made for quench, using an external standard, and for background counts. Fatty acid synthesis was calculated, without correction for isotope effects, as μg -atoms of H incorporated/h.

Student's *t* test was used to assess the statistical significance of differences between groups.

Insulin assay

The concentration of insulin in plasma was measured by radioimmunoassay using a kit with a rat insulin standard [RIA (U.K.) Ltd., Washington, Tyne and Wear, U.K.].

Results

Synthesis at 26 days of age

By 26 days of age the body weight of the obese mice was greater than that of the lean. The weight of interscapular brown adipose tissue was also increased in the obese ($190.1 \pm 11.5\text{mg}$ compared with $69.9 \pm 6.1\text{mg}$ for the lean mice; mean values \pm S.E.M., $n = 7$, $P < 0.001$), as was that of the liver and the epididymal fat-pads. Table 1 shows the rates of fatty acid synthesis obtained in tissues from the 26-day-old animals. The synthesis rate per g of tissue was higher in interscapular brown adipose

Table 1. Rates of fatty acid synthesis in tissues from lean and obese (*ob/ob*) mice at 26 days of age. Fatty acid synthesis was measured *in vivo* with $^3\text{H}_2\text{O}$; for full experimental details see the text. The values are means \pm S.E.M. for seven lean and seven obese animals. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$, compared with values for lean mice.

	Fatty acid synthesis (μg -atoms of H incorporated/h)			
	(per g of tissue)		(per total tissue)	
	Lean	Obese	Lean	Obese
Interscapular brown adipose tissue	113.1 ± 19.6	$252.8 \pm 30.1^{**}$	7.7 ± 1.1	$49.0 \pm 8.1^{***}$
Liver	48.3 ± 6.6	$151.4 \pm 12.4^{***}$	37.6 ± 5.4	$159.8 \pm 23.0^{***}$
Epididymal white adipose tissue	60.3 ± 12.9	$104.5 \pm 14.2^*$	5.1 ± 1.6	$28.3 \pm 4.3^{***}$
Subcutaneous white adipose tissue†	47.4 ± 8.6	$106.1 \pm 6.9^{***}$	3.1 ± 0.4	$19.5 \pm 2.2^{***}$
Carcass	15.8 ± 1.8	$35.5 \pm 5.4^{**}$	190.2 ± 25.4	$497.5 \pm 86.7^{**}$
Whole-body	—	—	243.6 ± 29.5	$754.2 \pm 95.7^{***}$

† The synthesis per total tissue for subcutaneous white adipose tissue is for the arbitrary sized sample removed.

tissue than in any other tissue, for both the lean and the obese mice. Synthesis was, however, greater in the obese than the lean mice for all tissues.

Rates of synthesis per g are of limited use, and may even be misleading, when comparing lean and obese animals, since the triacylglycerol content of their adipose tissues may differ considerably. Table 1 therefore also shows the synthesis per whole tissue, from which total body synthesis has been calculated. Whole-body synthesis in the obese was more than three times that in the lean, and this reflected increases in all the individual tissues. In the obese the total hepatic synthesis was 4.3 times that in the lean, whereas in the interscapular brown fat pads the total synthesis was 6.4 times the lean. In the lean mice 15.4% of whole-body synthesis could be attributed to the liver, compared with 21.2% in the obese mice. Interscapular brown adipose tissue accounted for 3.2% of whole-body synthesis in the lean, and 6.5% in the obese mice.

In assessing the overall importance of brown adipose tissue to whole-body synthesis, it should be noted that in the normal animal the interscapular pads only account for approximately one-quarter of the total dissectable tissue (Foster & Frydman, 1978).

Synthesis at 8 weeks of age

The differences in body weight and weight of individual tissues found between the lean and obese animals at 26 days of age were exaggerated by 8 weeks. At this later age the weight of the interscapular brown fat pads was 96.0 ± 8.3 and 352.4 ± 20.1 mg (mean values \pm S.E.M. for 7 animals; $P < 0.001$) for the lean and obese mice respectively. The rates of fatty acid synthesis at 8 weeks of age are shown in Table 2. In the lean mice the synthesis per g was still higher in brown adipose tissue than in other tissues, but the difference in rate between

brown fat and other tissues was greater at this age than at 26 days. Synthesis rates per g of tissue were also lower in the obese mice at 8 weeks than at 26 days of age, but the decrease in rate was greatest for brown adipose tissue. At 8 weeks of age brown adipose tissue was the only tissue to exhibit lower rates of synthesis per g in the obese than the lean mice.

Whole-body synthesis in the obese mice at 8 weeks was 3.5 times that in the lean. The proportion of whole-body synthesis attributable to the liver was 28.4% for the obese and 10.9% for the lean mice. Total hepatic synthesis in the obese mice was 9.2 times that in the lean mice, whereas the total synthesis in the epididymal fat-pads was greater by a factor of 18.6. In contrast, there was no significant difference between the lean and obese mice in the total synthesis in interscapular brown adipose tissue. The proportion of whole-body synthesis accounted for by interscapular brown fat in the lean mice was 3.0%, a value similar to that at 26 days of age. The equivalent value in the obese mice at 8 weeks was only 1.2%, which was much lower than at 26 days.

Developmental changes in synthesis in brown adipose tissue

The major change in fatty acid synthesis in the lean and obese animals between 26 days and 8 weeks of age was the marked decrease in rate in brown adipose tissue in the obese mice. In view of this, the developmental changes in synthesis in brown adipose tissue were investigated from the suckling period onwards. Fig. 1(a) shows the changes in weight of interscapular brown adipose tissue with age. At 14 days there was no difference in the weight of the tissue between the lean and obese mice, but by 21 days it was heavier in the obese animals. This difference in weight increased progressively as the animals became older, and is a

Table 2. *Rates of fatty acid synthesis in tissues from lean and obese (ob/ob) mice at 8 weeks of age*
Fatty acid synthesis was measured *in vivo* with $^3\text{H}_2\text{O}$; for full experimental details see the text. The values are means \pm S.E.M. for seven lean and seven obese animals. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$, compared with values for lean mice.

	Fatty acid synthesis (μg -atoms of H incorporated/h)			
	(per g of tissue)		(per total tissue)	
	Lean	Obese	Lean	Obese
Interscapular brown adipose tissue	102.5 ± 18.8	$42.2 \pm 6.9^{**}$	10.2 ± 2.4	14.8 ± 2.4
Liver	24.3 ± 3.6	$124.4 \pm 22.1^{***}$	37.3 ± 6.4	$342.9 \pm 71.0^{***}$
Epididymal white adipose tissue	10.6 ± 3.5	$27.9 \pm 4.8^{**}$	3.0 ± 0.6	$55.8 \pm 9.5^{***}$
Subcutaneous white adipose tissue†	11.5 ± 1.6	19.8 ± 4.2	1.6 ± 0.4	$17.5 \pm 3.5^{***}$
Carcass	11.5 ± 1.3	$18.6 \pm 2.9^*$	288.8 ± 32.9	$777.7 \pm 131.6^{**}$
Whole-body	—	—	340.9 ± 36.8	$1208.7 \pm 203.5^{**}$

† The synthesis per total tissue for subcutaneous white adipose tissue is for the arbitrary sized sample removed.

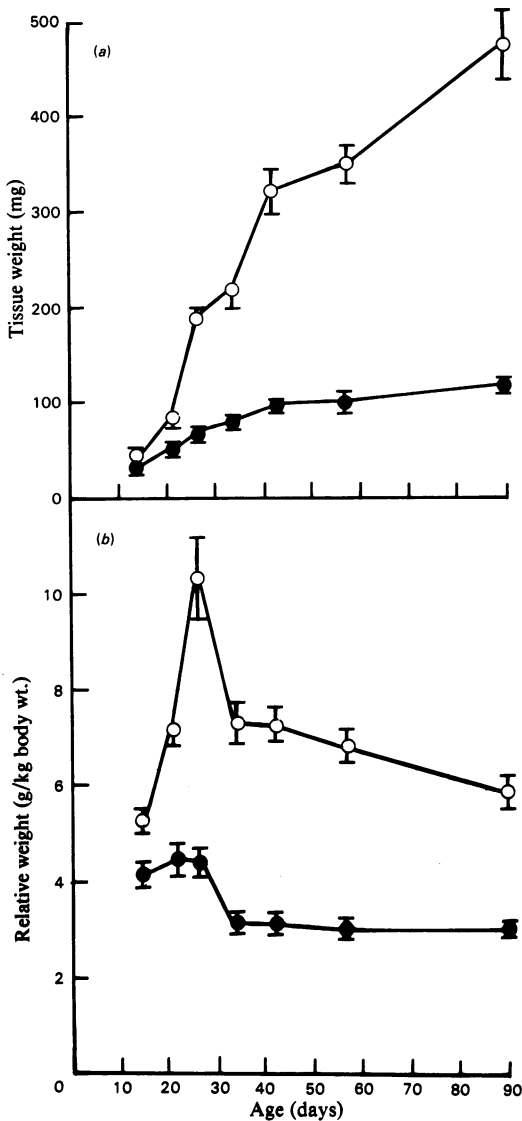


Fig. 1. Developmental changes in interscapular brown adipose tissue weight in lean (●) and obese (○) mice (a) Absolute weight; (b) proportion of body weight. The results are given as means \pm S.E.M. (indicated by the bars) for six or seven mice at each age. In both (a) and (b), the differences between the lean and obese animals were significant ($P < 0.001$) at all ages, except at 14 days.

reflection of the gradual accumulation of triacylglycerol in the obese mice; the extent to which the excess lipid deposition in interscapular brown adipose tissue of *ob/ob* mice may be due to the uptake of triacylglycerol from plasma, rather than to lipogenesis within the tissue is not clear. Fig. 1(b) shows the changes with age in the weight of

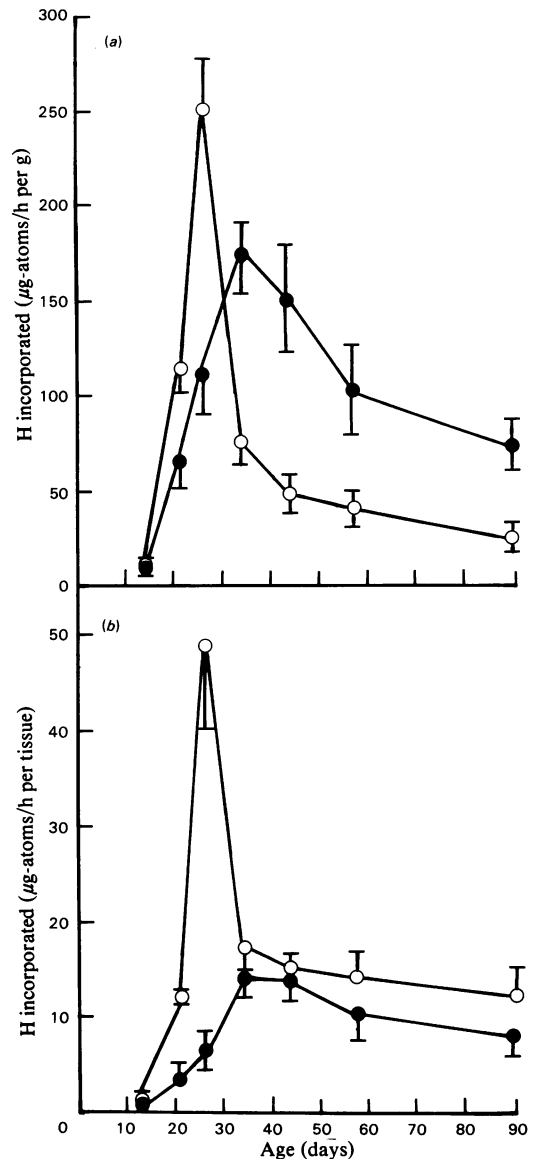


Fig. 2. Developmental changes in fatty acid synthesis in interscapular brown adipose tissue of lean (●) and obese (○) mice

For experimental details see the text. (a) synthesis/g tissue; (b) synthesis/total tissue. The results are given as means \pm S.E.M. (indicated by bars) for 6 or 7 mice at each age. In (a) the differences between the lean and obese animals were significant ($P < 0.05$, or better) at all ages, except at 14 days. In (b) the differences between lean and obese were significant ($P < 0.02$, or better) only at 21 and 26 days of age.

interscapular brown adipose tissue as a proportion of body weight. At all ages the relative weight of the tissue was greater in the obese than in the lean mice,

and this difference was most marked at 26 days of age. In the lean mice the relative weight was highest before and just after weaning.

At 14 days of age, fatty acid synthesis rates in interscapular brown adipose tissue were very low, both per g and per tissue, as reported previously for suckling animals (Trayhurn, 1981; Pillay & Bailey, 1982) and there was no difference between lean and obese mice (Fig. 2). Synthesis increased for both groups by weaning at 21 days, but at this age the rate had become greater in the obese than in the lean mice. By 26 days the rates had increased further, and, as shown in Table 1, were much higher in the obese mice, particularly when expressed per tissue. Between 26 and 35 days of age there was a further rise in synthesis in the lean mice, and the peak rate in these animals occurred at 35 days. In contrast, in the obese mice there was a sharp decrease in synthesis rate between 26 and 35 days. By 35 days the rate per g in the obese mice was less than half that in the lean, and there was no longer any significant difference between the two groups in the rate per tissue. Between 35 and 90 days, synthesis rates gradually declined in both the lean and obese mice, but throughout this period the rate per tissue was not significantly different in the two groups.

Discussion

In the first part of the present study the rate of fatty acid synthesis in interscapular brown adipose tissue of obese (*ob/ob*) mice was determined and a comparison made with the rates of synthesis in other tissues and in normal lean animals. At 26 days of age, shortly after weaning and just before the development of hyperphagia (Lin *et al.*, 1977; S. W. Mercer, unpublished observations), fatty acid synthesis rates in brown adipose tissue of the obese mice were higher than those in other lipogenic tissues; the rates were also higher than those found in the lean mice. However, by 8 weeks of age, when hyperphagia was well-established, synthesis rates in brown adipose tissue of the obese mice were lower than those in the liver, and lower than those in brown fat of the lean mice.

The second part of the present study has shown that there is a rapid increase in fatty acid synthesis in brown adipose tissue at and just after the time of weaning on to a low-fat/high-carbohydrate diet, and this phenomenon is similar to that observed for white adipose tissue and the liver (Le Marchand-Brustel & Jeanrenaud, 1978; Rath & Thenen, 1980; Gandermer *et al.*, 1982). Since fatty acid synthesis in brown adipose tissue can be suppressed on diets rich in long-chain fatty acids (van den Brandt & Trayhurn, 1981), the post-weaning increase in lipogenesis in the tissue could presumably be inhibited by the provision of a high-fat diet, at least

in lean animals. The rise at weaning in fatty acid synthesis in brown fat was much more marked in the obese than in the lean mice. This is likely to be due to the hyperinsulinaemia that begins to develop in the *ob/ob* mutant before weaning (Godbole *et al.*, 1980; Dubuc, 1981), since fatty acid synthesis in brown adipose tissue has been shown to be insulin-sensitive (McCormack & Denton, 1977; Agius & Williamson, 1980; McCormack, 1982). At 26 days of age in the present experiments, when synthesis in brown adipose tissue in the obese mice was at a peak, the plasma insulin concentration was 13 times that found in the lean siblings (26.1 ± 3.5 compared with 2.0 ± 0.7 ng/ml, for obese and lean mice respectively; mean values \pm S.E.M. for five mice in each group).

At 8 weeks of age fatty acid synthesis rates in liver, white adipose tissue (both epididymal and subcutaneous) and the carcass remained much higher in the obese animals than in the lean ones. This was in marked contrast with interscapular brown adipose tissue, which showed a sharp fall in synthesis after week 4 of life, suggesting that this tissue develops insulin resistance much more rapidly than other lipogenic tissues in the *ob/ob* mouse. At 8 weeks of age the plasma insulin concentration was approx. 17 times higher in the obese than in the lean mice (37.1 ± 6.7 compared with 2.2 ± 0.6 ng/ml, for obese and lean mice respectively; mean values \pm S.E.M. for six mice in each group).

In normal rats and mice fed a low-fat/high-carbohydrate diet there appears to be a direct relationship in brown adipose tissue between the rate of thermogenesis and the rate of lipogenesis (Trayhurn, 1979, 1981). However, from the present work no such clear relationship between the two processes is apparent in the tissue from the *ob/ob* mouse. Nevertheless, it is of interest that from week 5 of life brown adipose tissue is the only major lipogenic tissue in the *ob/ob* mutant, with its reduced thermogenesis, that does not show elevated rates of fatty acid synthesis. The rapid development of insulin resistance in brown adipose tissue of the obese mice might therefore be the result of an attempt to more closely relate the rate of fatty acid synthesis in the tissue, and the rate of glucose uptake, to the rate of thermogenesis.

Recently, Lavau *et al.* (1982) have reported that in Zucker rats, aged 30 days, the rate of fatty acid synthesis in interscapular brown adipose tissue is substantially elevated compared with lean animals. It was consequently suggested that brown adipose tissue might make a major contribution to the development of obesity in the Zucker rat, by virtue of the export of fatty acids for storage in white adipose tissue. However, since fatty acid synthesis rates were obtained for one age only, it is not clear whether the hyperlipogenesis in brown fat observed at 30 days of age is sustained over a long period.

Our results for the *ob/ob* mouse, with the transitory hyperlipogenesis in brown adipose tissue with a peak at 26 days of age, demonstrate that conclusions based on a single time point taken soon after weaning could well be erroneous. In the case of the *ob/ob* mouse the transitory nature of the elevated fatty acid synthesis rates in interscapular brown adipose tissue, together with the reduced quantitative importance of the tissue in whole-body synthesis once hyperphagia has developed, makes it very unlikely that brown fat plays a major role in the synthesis of fatty acids for storage elsewhere. Furthermore, the peak in fatty acid synthesis in brown adipose tissue of the obese mice at 26 days of age corresponds to the time at which the relative weight of the tissue is at a maximum (Fig. 1b). This suggests that most of the extra fatty acids being synthesized by brown adipose tissue in the obese mice during the post-weaning surge may be stored within the tissue.

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